

Ford, V.  
09/825769

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FILE 'REGISTRY' ENTERED AT 11:03:44 ON 31 OCT 2002

E PERTUSSIS TOXIN/CN 5

L1 12 S E3-E14  
E BORDETELLA PERTUSSIS CYSTEINE DESULFINASE/CN 5

L2 1 S E2

L3 13 S L1 OR L2  
E CYSTEINE DESULFINASE/CN 5

FILE 'HCAPLUS' ENTERED AT 11:05:49 ON 31 OCT 2002

L1 12 SEA FILE=REGISTRY ABB=ON PLU=ON ("PERTUSSIS TOXIN"/CN  
OR "PERTUSSIS TOXIN (BORDETELLA PERTUSSIS 10536 CLONE  
J-169-1 S1 SUBUNIT)"/CN OR "PERTUSSIS TOXIN (BORDETELLA  
PERTUSSIS 10536 CLONE J-169-1 S2 SUBUNIT)"/CN OR  
"PERTUSSIS TOXIN (BORDETELLA PERTUSSIS 10536 CLONE  
J-169-1 S3 SUBUNIT)"/CN OR "PERTUSSIS TOXIN (BORDETELLA  
PERTUSSIS TOXIN SUBUNIT S1 VARIANT 1)"/CN OR "PERTUSSIS  
TOXIN (BORDETELLA PERTUSSIS TOXIN SUBUNIT S1 VARIANT  
2)"/CN OR "PERTUSSIS TOXIN (SYNTHETIC BORDETELLA  
BRONCHISEPTICA-BORDETELLA PERTUSSIS SUBUNIT S1)"/CN OR  
"PERTUSSIS TOXIN (SYNTHETIC BORDETELLA BRONCHISEPTICA-BOR  
DETELLA PERTUSSIS SUBUNIT S2)"/CN OR "PERTUSSIS TOXIN  
(SYNTHETIC BORDETELLA BRONCHISEPTICA-BORDETELLA PERTUSSIS  
SUBUNIT S3)"/CN OR "PERTUSSIS TOXIN (SYNTHETIC BORDETELL  
A BRONCHISEPTICA-BORDETELLA PERTUSSIS SUBUNIT S4)"/CN OR  
"PERTUSSIS TOXIN (SYNTHETIC BORDETELLA BRONCHISEPTICA-BOR  
DETELLA PERTUSSIS SUBUNIT S5)"/CN OR "PERTUSSIS TOXIN  
(SYNTHETIC)"/CN)

L2 1 SEA FILE=REGISTRY ABB=ON PLU=ON "BORDETELLA BRONCHISEPT  
ICA"/CN

L3 13 SEA FILE=REGISTRY ABB=ON PLU=ON L1 OR L2

L4 9821 SEA FILE=HCAPLUS ABB=ON PLU=ON L3 OR PT(10A)TOXIN OR  
PERTUSSIS TOXIN

~~L5~~ 1 SEA FILE=HCAPLUS ABB=ON PLU=ON L4 AND (CYSTEINE(S) (DESU  
LFINASE OR DESULPHINASE OR DE(W) (SULFINASE OR SULPHINASE)  
) OR PTA3254 OR PTA(5A)3254)

L1 12 SEA FILE=REGISTRY ABB=ON PLU=ON ("PERTUSSIS TOXIN"/CN  
OR "PERTUSSIS TOXIN (BORDETELLA PERTUSSIS 10536 CLONE  
J-169-1 S1 SUBUNIT)"/CN OR "PERTUSSIS TOXIN (BORDETELLA  
PERTUSSIS 10536 CLONE J-169-1 S2 SUBUNIT)"/CN OR  
"PERTUSSIS TOXIN (BORDETELLA PERTUSSIS 10536 CLONE  
J-169-1 S3 SUBUNIT)"/CN OR "PERTUSSIS TOXIN (BORDETELLA  
PERTUSSIS TOXIN SUBUNIT S1 VARIANT 1)"/CN OR "PERTUSSIS  
TOXIN (BORDETELLA PERTUSSIS TOXIN SUBUNIT S1 VARIANT  
2)"/CN OR "PERTUSSIS TOXIN (SYNTHETIC BORDETELLA  
BRONCHISEPTICA-BORDETELLA PERTUSSIS SUBUNIT S1)"/CN OR  
"PERTUSSIS TOXIN (SYNTHETIC BORDETELLA BRONCHISEPTICA-BOR  
DETELLA PERTUSSIS SUBUNIT S2)"/CN OR "PERTUSSIS TOXIN  
(SYNTHETIC BORDETELLA BRONCHISEPTICA-BORDETELLA PERTUSSIS  
SUBUNIT S3)"/CN OR "PERTUSSIS TOXIN (SYNTHETIC BORDETELL  
A BRONCHISEPTICA-BORDETELLA PERTUSSIS SUBUNIT S4)"/CN OR  
"PERTUSSIS TOXIN (SYNTHETIC BORDETELLA BRONCHISEPTICA-BOR  
DETELLA PERTUSSIS SUBUNIT S5)"/CN OR "PERTUSSIS TOXIN  
(SYNTHETIC)"/CN)

L2 1 SEA FILE=REGISTRY ABB=ON PLU=ON "BORDETELLA BRONCHISEPT  
ICA"/CN

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L3 13 SEA FILE=REGISTRY ABB=ON PLU=ON L1 OR L2  
L4 9821 SEA FILE=HCAPLUS ABB=ON PLU=ON L3 OR PT(10A)TOXIN OR  
PERTUSSIS TOXIN  
L6 466 SEA FILE=HCAPLUS ABB=ON PLU=ON L4 (5A) (PROD# OR  
PRODUCTION OR PRODUCING OR PRODUCE# OR MANUF? OR PREP?  
OR GROW?)  
L7 47 SEA FILE=HCAPLUS ABB=ON PLU=ON L6 AND (MUTAT? OR  
MUTANT OR MUTAGEN? OR POLYMORPH? OR POLY MORPH?)  
~~L8~~ 3 SEA FILE=HCAPLUS ABB=ON PLU=ON L7 AND (METHOD OR  
TECHNIQUE)

~~PP9~~ 3 L5 OR L8

L9 ANSWER 1 OF 3 HCAPLUS COPYRIGHT 2002 ACS  
ACCESSION NUMBER: 2002:10698 HCAPLUS  
DOCUMENT NUMBER: 136:80864  
TITLE: Method of creating a high yield  
pertussis vaccine production strain of  
Bordatella bronchiseptica  
INVENTOR(S): Merkel, Tod J.; Keith, Jerry M.; Yang, Xiaoming  
PATENT ASSIGNEE(S): Government of the United States of America, as  
Represented by the Secretary, Department of  
Health and Human Services, USA  
SOURCE: PCT Int. Appl., 35 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002000895	A2	20020103	WO 2001-US20356	20010626
WO 2002000895	A3	20020530		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 2001071495	A5	20020108	AU 2001-71495	20010626
PRIORITY APPLN. INFO.:			US 2000-214072P P	20000626
			WO 2001-US20356 W	20010626

AB The present invention provides a **method** to genetically engineer a pertussis vaccine prodn. strain of *Bordetella bronchiseptica* that **produces a pertussis toxin** in high yield and good quality. The **method** includes introducing a plasmid contg. a DNA encoding antibiotic resistance gene into a *Bordetella bronchiseptica* strain, selecting for isolates in which the antibiotic resistant gene is recombinantly incorporated into the chromosome in place of the *Bordetella*

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bronchiseptica toxin gene, introducing a plasmid contg. DNA encoding subunits of the *Bordetella pertussis* toxins into the *Bordetella bronchiseptica* isolates, and, selecting for isolates in which DNA encoding *Bordetella pertussis* toxin subunit is recombinantly incorporated into the chromosome, the resulting cells producing the *Bordetella pertussis* toxin . The invention also provides a method for creating a *Bordetella bronchiseptica* cell line which produces a *Bordetella pertussis* toxin and does not express filamentous hemagglutinin.

L9 ANSWER 2 OF 3 HCAPLUS COPYRIGHT 2002 ACS  
ACCESSION NUMBER: 2001:747833 HCAPLUS  
DOCUMENT NUMBER: 135:302952  
TITLE: Improved method for the production of bacterial toxins  
INVENTOR(S): Blake, Milan S.; Bogdan, John A., Jr.; Nazario-Larrieu, Javier  
PATENT ASSIGNEE(S): Baxter International Inc., USA; Baxter Healthcare S.A.  
SOURCE: PCT Int. Appl., 46 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001074862	A2	20011011	WO 2001-US10938	20010404
WO 2001074862	A3	20021003		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 2002061555	A1	20020523	US 2001-825770	20010404
PRIORITY APPLN. INFO.:			US 2000-194478P	P 20000404
			US 2000-194482P	P 20000404

AB Methods and compns. are provided for the enhanced prodn. of bacterial toxins in large-scale cultures. Specifically, methods and compns. for reducing bacterial toxin expression inhibitors are provided including, but not limited to, addn. of toxin expression inhibitor binding compds., culture media having reduced concns. of toxin inhibitor metabolic precursors and genetically modified toxigenic bacteria lacking enzymes required to metabolize the toxin inhibitor metabolic precursors.

L9 ANSWER 3 OF 3 HCAPLUS COPYRIGHT 2002 ACS  
ACCESSION NUMBER: 1994:75437 HCAPLUS  
DOCUMENT NUMBER: 120:75437  
TITLE: Genetic detoxification of pertussis toxin for

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vaccine  
INVENTOR(S): Klein, Michel H.; Boux, Heather A.; Cockle, Stephen A.; Loosmore, Sheena M.; Zealey, Gavin R.  
PATENT ASSIGNEE(S): Connaught Laboratories Ltd., Can.  
SOURCE: U.S., 46 pp. Cont-in-part of U.S. 5,085,862.  
CODEN: USXXAM  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 2  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5244657	A	19930914	US 1990-589423	19900928
US 5085862	A	19920204	US 1988-275376	19881123
US 5221618	A	19930622	US 1991-767837	19910930
US 5332583	A	19940726	US 1991-788314	19911105
US 5358868	A	19941025	US 1991-788313	19911105
US 5433945	A	19950718	US 1992-979798	19921120
PRIORITY APPLN. INFO.:			GB 1987-27489	A 19871124
			US 1988-275376	A2 19881123
			US 1990-589423	A3 19900928

AB A method is described for the prepn. of a safe, immunogenic, and efficacious vaccine for protection against pertussis. Specific functional sites of pertussis toxin have been identified, and, using this information, defined mutant holotoxins have been produced by site-directed mutagenesis of the toxin gene. A no. of these holotoxin analogs are detoxified, retain an immunodominant S1 epitope, are immunogenic, and are protective in the std. pertussis vaccine potency test in mice. The site of interaction of the S1 subunit with NAD was also detd.

(FILE'D MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO, TOXCENTER' ENTERED AT 11:20:47 ON 31 OCT 2002)

L10 3 S L5  
L11 14 S L8  
~~L12~~ 16 S L10 OR L11  
~~L13~~ L1-DUP REM L12 (5 DUPLICATES REMOVED)

L13 ANSWER 1 OF 11 WPIDS (C) 2002 THOMSON DERWENT  
ACCESSION NUMBER: 2002-271039 [32] WPIDS  
CROSS REFERENCE: 1993-019976 [03]  
DOC. NO. CPI: C2002-080491  
TITLE: Genetically modified *Bordetella* strain having genome characterized by the absence of a filamentous hemagglutinin gene, and presence of a genetically detoxified mutant TOX gene, useful for producing antigen for vaccines.

DERWENT CLASS: B04 D16  
INVENTOR(S): KLEIN, M; LOOSMORE, S; YACOOB, R; ZEALEY, G  
PATENT ASSIGNEE(S): (AVET) AVENTIS PASTEUR LTD  
COUNTRY COUNT: 16  
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
EP 1184459	A2	20020306 (200232)*	EN	28	

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R: AT BE CH DE DK ES FR GB GR IT LI LU MC NL PT SE

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
EP 1184459	A2 Div ex	EP 1992-306474 EP 2001-111022	19920715 19920715

FILING DETAILS:

PATENT NO	KIND	PATENT NO
EP 1184459	A2 Div ex	EP 523976

PRIORITY APPLN. INFO: GB 1991-15332 19910716

AN 2002-271039 [32] WPIDS

CR 1993-019976 [03]

AB EP 1184459 A UPAB: 20020521

NOVELTY - Genetically-modified *Bordetella* (B) strain having a genome:

(a) characterized by absence of filamentous hemagglutinin gene (FHA), and presence of genetically detoxified **mutant** TOX gene; or

(b) containing a copy of a hybrid gene comprising pertactin (PRN) structural gene under the regulation of the FHA promoter, is new. (B) strain comprising hybrid gene is identified as *B.pertussis* strain 591-473.

DETAILED DESCRIPTION - Genetically-modified *Bordetella* (B) strain (I) which is *B.pertussis* strain 591-473 (ATCC acc.No.55321) comprises a genome:

(a) from which the FHA gene is absent, and which contains a genetically-detoxified **mutant** TOX gene; or

(b) containing a copy of hybrid gene comprising a PRN structural gene under the regulation of the FHA promoter.

An INDEPENDENT CLAIM is also included for a vaccine against *Bordetella* infection comprising as a single component or as one component of a multicomponent vaccine, a killed (I).

ACTIVITY - Antibacterial.

No suitable data given.

MECHANISM OF ACTION - Vaccine.

USE - (I) is useful for producing an antigen which involves culturing (I) in a culture medium to effect expression of protein encoded by genes present in the strain. The expressed protein is extracted from the culture medium and detoxified for vaccine use (all claimed). (I) is useful in producing a whole cell or defined component vaccine against (B), particularly whooping cough.

ADVANTAGE - Optimal production of antigens for new vaccine formulations can be achieved using (I). Undesirable genes may be removed, expression of antigens produced in limiting amounts may be enhanced, and purification procedures are simplified.

DESCRIPTION OF DRAWING(S) - The drawing shows plasmids used to produce copy-number altered strains.

Dwg.1A/7

L13 ANSWER 2 OF 11 WPIDS (C) 2002 THOMSON DERWENT DUPLICATE 1  
ACCESSION NUMBER: 2002-010777 [01] WPIDS  
DOC. NO. CPI: C2002-002634

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TITLE: Enhancing production of bacterial toxins comprises eliminating or reducing toxin expression inhibitors formed by toxin producing bacteria by adding at least one soluble metal salt that forms an insoluble complex with sulfate.

DERWENT CLASS: B04 D16

INVENTOR(S): BLAKE, M S; BOGDAN, J A; NAZARIO-LARRIEU, J

PATENT ASSIGNEE(S): (BAXT-N) BAXTER HEALTHCARE SA; (BAXT) BAXTER INT INC; (BLAK-I) BLAKE M S; (BOGD-I) BOGDAN J A; (NAZA-I) NAZARIO-LARRIEU J

COUNTRY COUNT: 95

PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001074862	A2	20011011 (200201)*	EN	46	
RW:	AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW				
W:	AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW				
AU 2001053134 A	20011015 (200209)				
US 2002061555 A1	20020523 (200239)				

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001074862	A2	WO 2001-US10938	20010404
AU 2001053134	A	AU 2001-53134	20010404
US 2002061555	A1 Provisional	US 2000-194482P	20000404
		US 2001-825770	20010404

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2001053134 A	Based on	WO 200174862

PRIORITY APPLN. INFO: US 2000-194482P 20000404; US 2000-194478P  
20000404; US 2001-825770 20010404

AN 2002-010777 [01] WPIDS

AB WO 200174862 A UPAB: 20020105

NOVELTY - Enhancing production of bacterial toxins comprises eliminating or reducing toxin expression inhibitors formed by toxin producing bacteria.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a **method** of cultivating *Bordetella pertussis* in the presence of one or more soluble metal salts that form a substantially insoluble complex with sulfate;

(2) a **method** of making a culture medium that supports *B. pertussis* growth and prevents or decreases inhibition of **pertussis toxin (PT)** expression by sulfate, by admixing a *B. pertussis* culture medium with one or more soluble metal salts that form a substantially insoluble complex with

sulfate;

(3) a culture medium that supports the growth of *B. pertussis* comprising one or more soluble metal salts that form a substantially insoluble complex with sulfate, where the amount prevents or reduces the inhibition of PT expression by sulfate;

(4) methods of producing PT comprising growing *B. pertussis* in a medium comprising a soluble metal salt that forms an insoluble complex with sulfate, and isolating the PT from the culture medium;

(5) a *B. pertussis cysteine desulfinase knockout mutant*;

(6) a method of enhanced production of PT by cultivating *B. pertussis cysteine desulfinase knockout mutant* where an enhanced amount of PT produced is compared to when a non-*cysteine desulfinase knockout mutant* is employed;

(7) a peptide comprising the amino acid sequence GGGDGGSFSGFGDGSFSGFG-OH (I);

(8) a method of isolating a bacterial toxin from a mixture by preparing a peptide affinity column where the peptide is (I), comprising:

(a) adding the mixture containing the bacterial toxin to the peptide affinity column, where the bacterial toxin binds to the peptide;

(b) releasing the bound bacterial toxin from the peptide; and  
(c) collecting the isolated bacterial toxin.

USE - The method is useful for increasing production of *pertussis toxin* by reducing or eliminating the accumulation of *Bordetella* species toxin expression inhibitors.

ADVANTAGE - Compared with previous methods of producing PT, e.g. growing *B. pertussis* in a stationary culture which is labor intensive, or cultivation on a fermentation scale which requires vortex stirring and surface modification, the new method results to increased or higher production of toxins.

Dwg.0/8

L13 ANSWER 3 OF 11 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 2002:176480 BIOSIS

DOCUMENT NUMBER: PREV200200176480

TITLE: Identification and characterization of a *cysteine desulfinase* gene in *Bordetella pertussis*.

AUTHOR(S): Yuan, W. (1); Bogdan, J. A. (1); Blake, M. S. (1)

CORPORATE SOURCE: (1) Baxter Healthcare Corporation, Columbia, MD USA

SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (2001) Vol. 101, pp. 87. <http://www.asmusa.org/mtgsrc/generalmeeting.htm>. print.

Meeting Info.: 101st General Meeting of the American Society for Microbiology Orlando, FL, USA May 20-24, 2001

ISSN: 1060-2011.

DOCUMENT TYPE: Conference

LANGUAGE: English

AB Many studies have shown that sulfate ions inhibit the production of *pertussis toxin* (Ptx). We have shown that sulfur containing amino acids, methionine and *cysteine*,

accumulate during fermentation in the late exponential phase of bacterial growth in concert with the appearance of sulfate anion in the media. Ptx expression begins to wane approximately at the same time as measurable sulfate anion can be detected. Our hypothesis is that the accumulation of sulfate anion acts as a natural negative feedback inhibitor of Ptx expression. An NIFS-like protein of *E. coli* has been cloned and reported to have **cysteine desulfinase** activity, removing the sulfate ion from **cysteine**. We have identified a similar **cysteine desulfinase** (dsf) gene on a 1.2 Kb DNA fragment from a *B. pertussis* genomic library. The DNA sequence of the region showed an ORF having a striking sequence homology at the translated protein level with the dsf gene of *E. coli*. Analysis by Southern blotting, using the full-length gene as the probe, demonstrated that only a single copy was present in the genome of three different *B. pertussis* strains. To determine the expression pattern of the **desulfinase** gene in our *B. pertussis* strain, we performed RT-PCR on total RNA extracted from the cell pellets harvested at different time points during fermentation. These studies showed that 'cdsf' transcription increased at 10 hours during fermentation, which correlated well with the observed increase of sulfate in the media.

L13 ANSWER 4 OF 11 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.  
DUPLICATE 2

ACCESSION NUMBER: 1997:316519 BIOSIS  
 DOCUMENT NUMBER: PREV199799607007  
 TITLE: Endocytosis and retrograde transport of pertussis toxin to the Golgi complex as a prerequisite for cellular intoxication.  
 AUTHOR(S): El Baya, Ali; Linnemann, Ruth; Von Olleschik-Elbheim, Lars; Robenek, Horst; Schmidt, M. Alexander (1)  
 CORPORATE SOURCE: (1) Inst. Infektioologie, ZMBE, Westfaelische Wilhelms-Univ., Von-Esmarch-Str. 56, D-48149 Muenster Germany  
 SOURCE: European Journal of Cell Biology, (1997) Vol. 73, No. 1, pp. 40-48.  
 ISSN: 0171-9335.

DOCUMENT TYPE: Article

LANGUAGE: English

AB The uptake mechanism of pertussis toxin (PT) in CHO and insulin-producing HIT-T15 cells was studied. By electron microscopy after direct labeling of the toxin with gold particles, PT was found to be taken up by receptor-mediated endocytosis. The presence of active pertussis toxin in the Golgi complex was shown by subcellular fractionation. The importance of the Golgi localization of pertussis toxin for the S1-dependent ADP-ribosylation of G-proteins was investigated employing Brefeldin A (BFA) treatment to disrupt Golgi structures. Treatment with Brefeldin A completely blocked the pertussis toxin mediated ADP-ribosylation of cellular G-proteins in CHO and HIT-T15 cells, whereas the BFA-resistant MDCK cells were not protected. A mutant CHO cell line (V24.1) exhibiting a temperature-sensitive Golgi complex could be protected when grown at restrictive conditions. These results strongly indicate that retrograde transport to the Golgi network is a necessary prerequisite for pertussis toxin mediated ADP-ribosylation of G-proteins and thus also for cellular intoxication.

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L13 ANSWER 5 OF 11 MEDLINE  
ACCESSION NUMBER: 97014557 MEDLINE  
DOCUMENT NUMBER: 97014557 PubMed ID: 8861392  
TITLE: Transfer of a pertussis toxin expression locus to isogenic bvg-positive and bvg-negative strains of *Bordetella bronchiseptica* using an in vivo technique.  
AUTHOR: Smith A M; Walker M J  
CORPORATE SOURCE: Department of Biological Sciences, University of Wollongong, NSW, Australia.  
SOURCE: MICROBIAL PATHOGENESIS, (1996 May) 20 (5) 263-73.  
PUB. COUNTRY: ENGLAND: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199704  
ENTRY DATE: Entered STN: 19970507  
Last Updated on STN: 19970507  
Entered Medline: 19970429

AB *Bordetella pertussis* is the causative agent of whooping cough, a contagious childhood respiratory disease, increasing public concern over the safety of current whole-cell vaccines has led to decreased immunization rates and a subsequent increase in the incidence of the disease. The preparation of safer vaccines is at present concentrated on the production of detoxified virulence factors such as pertussis toxin (PT) for inclusion in acellular vaccine preparations. A permanently avirulent *Bordetella bronchiseptica* strain was previously engineered to constitutively produce PT. An in vivo cloning technique, based on the principles of conjugal mating and chromosome transfer was employed to transfer the PT expression locus of this strain to virulent and avirulent strains of *B. bronchiseptica*. This transfer was confirmed by Southern hybridization. An analysis of PT secretion in isogenic virulent and avirulent strains of *B. bronchiseptica* revealed that the PT produced was cell-associated and not secreted to the growth medium. This evidence suggests that *B. bronchiseptica* does not possess functional PT secretion (ptl) genes. Therefore, to achieve a PT expression and secretion system suitable for vaccine purposes in *Bordetella bronchiseptica*, functional ptl genes of *B. pertussis* are also required.

L13 ANSWER 6 OF 11 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.  
DUPLICATE 3  
ACCESSION NUMBER: 1995:361135 BIOSIS  
DOCUMENT NUMBER: PREV199598375435  
TITLE: Identification of a *Bordetella pertussis* regulatory factor required for transcription of the pertussis toxin operon in *Escherichia coli*.  
AUTHOR(S): Deshazer, David; Wood, Gwendolyne E.; Friedman, Richard L. (1)  
CORPORATE SOURCE: (1) Dep. Microbiol. Immunol., Univ. Ariz., Tucson, AZ 85724 USA  
SOURCE: Journal of Bacteriology, (1995) Vol. 177, No. 13, pp. 3801-3807.  
ISSN: 0021-9193.  
DOCUMENT TYPE: Article

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LANGUAGE: English

AB Transcription of the pertussis toxin operon (ptx) is positively regulated in *Bordetella pertussis* by the bvgAS locus. However, a ptx-lacZ transcriptional fusion in *Escherichia coli* cannot be activated by bvgAS in trans. This suggests that an additional factor(s) is required for transcription of ptx. A gene encoding a Bvg accessory factor (Baf) was identified by its ability to activate an *E. coli* ptx-lacZ fusion in the presence of bvgAS. The expression of ptr-lacZ was decreased by the addition of 40 MM MgSO<sub>4</sub>, a compound that also modulates ptx expression in *B. pertussis*. Baf alone did not activate expression of an *E. coli* fhaB-lacZ fusion, nor did it increase expression of jhaB-lacZ in trans with bvgAS. The gene encoding Baf was localized, sequenced, and found to produce a novel 28-kDa protein. Sequences homologous to *B. pertussis* baf were identified in *Bordetella bronchiseptica* and *Bordetella parapertussis* but not in *Bordetella avium*. When an additional copy of baf was integrated into the chromosome of BC75, a *B. pertussis* mutant that produces a low level of pertussis toxin, pertussis toxin production was partially complemented in the cointegrate strain.

L13 ANSWER 7 OF 11 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

DUPLICATE 4

ACCESSION NUMBER: 1992:141704 BIOSIS

DOCUMENT NUMBER: BA93:75929

TITLE: CONSTRUCTION OF BORDETELLA-PERTUSSIS STRAINS THAT OVERPRODUCE GENETICALLY INACTIVATED PERTUSSIS TOXIN.

AUTHOR(S): ZEALEY G R; LOOSMORE S M; YACOOB R K; COCKLE S A; HERBERT A B; MILLER L D; MACKAY N J; KLEIN M H

CORPORATE SOURCE: CONNAUGHT CENTRE BIOTECHNOLOGY RESEARCH, 1755 STEELES AVENUE WEST, WILLOWDALE, ONTARIO, CAN. M2R 3T4.

SOURCE: APPL ENVIRON MICROBIOL, (1992) 58 (1), 208-214.  
CODEN: AEMIDF. ISSN: 0099-2240.

FILE SEGMENT: BA; OLD

LANGUAGE: English

AB Nontoxic analogs of pertussis toxin (PT), produced by in vitro mutagenesis of the tox operon, are immunogenic and protective against infection by *Bordetella pertussis*. The moderate levels of PT production by *B. pertussis*, however, make it the limiting antigen in the formulation of multicomponent, acellular, recombinant whooping cough vaccines. To increase production of the highly detoxified Lys9Gly129 PT analog by *B. pertussis*, additional copies of the mutated tox operon were integrated into the bacterial chromosome at the tox of fha locus by unmarked allelic exchange. Recombinant strains produced in this way secreted elevated levels of the PT analog proportional to gene dosage. The strains were stable during 10-liter fermentations, and yields of up to 80 mg of PT analog per liter were obtained under production-scale conditions. The nontoxic analog was purified and shown to be indistinguishable from material obtained from a *B. pertussis* strain that contained only a single copy of the toxLys9Gly9 operon. Such strains are therefore suitable for large-scale, industrial production of an acellular whooping cough vaccine containing a genetically detoxified PT analog.

L13 ANSWER 8 OF 11 WPIDS (C) 2002 THOMSON DERWENT

ACCESSION NUMBER: 1989-186481 [26] WPIDS

09/825769

CROSS REFERENCE: 1996-041582 [05]  
DOC. NO. CPI: C1989-082452  
TITLE: Immuno-protective, genetically-detoxified pertussis toxin and vaccine - with aminoacid substitution(s) or deletion(s) produced by site-directed mutagenesis of toxin gene.  
DERWENT CLASS: B04 D16  
INVENTOR(S): BOUX, H A; COCKLE, S A; KLEIN, M H; LOOSMORE, S M; ZEALEY, G R  
PATENT ASSIGNEE(S): (CONN-N) CONNAUGHT LABS LTD; (CONN-N) CONNAUGHT LAB LTD  
COUNTRY COUNT: 15  
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
EP 322115	A	19890628 (198926)*	EN	42	
R: AT BE	CH DE ES FR GB GR IT LI LU NL SE				
JP 02002383	A	19900108 (199007)			
US 5085862	A	19920204 (199208)			
US 5221618	A	19930622 (199326)		37	
US 5244657	A	19930914 (199338)		46	
US 5332583	A	19940726 (199429)		45	
US 5358868	A	19941025 (199442)		45	
US 5433945	A	19950718 (199534)		47	
EP 322115	B1	19960306 (199614)	EN	49	
R: AT BE	CH DE ES FR GB GR IT LI LU NL SE				
DE 3855072	G	19960411 (199620)			
ES 2088778	T3	19960916 (199643)			
JP 2714068	B2	19980216 (199812)		37	
EP 322115	B2	20010228 (200113)	EN		
R: AT BE	CH DE ES FR GB GR IT LI LU NL SE				

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
EP 322115	A	EP 1988-311133	19881124
JP 02002383	A	JP 1988-297152	19881124
US 5085862	A	US 1988-275376	19881123
US 5221618	Div ex	US 1988-275376	19881123
		US 1991-767837	19910930
US 5244657	A	US 1988-275376	19881123
		US 1990-589423	19900928
US 5332583	A	US 1988-275376	19881123
	Div ex	US 1989-589423	19890928
		US 1991-788314	19911105
US 5358868	A	US 1988-275376	19881123
	Div ex	US 1990-589423	19900928
		US 1991-788313	19911105
US 5433945	A	US 1988-275376	19881123
	Div ex	US 1990-589423	19900928
		US 1992-979798	19921120
EP 322115	B1	EP 1988-311133	19881124
DE 3855072	G	DE 1988-3855072	19881124
		EP 1988-311133	19881124
ES 2088778	T3	EP 1988-311133	19881124
JP 2714068	B2	JP 1988-297152	19881124

09/825769

EP 322115	B2	Related to	EP 1988-311133	19881124
			EP 1995-111215	19881124

FILING DETAILS:

PATENT NO	KIND	PATENT NO
US 5221618	A Div ex	US 5085862
US 5244657	A CIP of	US 5085862
US 5332583	A CIP of Div ex	US 5085862 US 5244657
US 5358868	A CIP of Div ex	US 5085862 US 5244657
US 5433945	A CIP of Div ex	US 5045862 US 5244657
DE 3855072	G Based on	EP 322115
ES 2088778	T3 Based on	EP 322115
JP 2714068	B2 Previous Publ.	JP 02002383
EP 322115	B2 Related to	EP 688868

PRIORITY APPLN. INFO: GB 1987-27489 19871124

AN 1989-186481 [26] WPIDS

CR 1996-041582 [05]

AB EP 322115 A UPAB: 20010307

An immunoprotective, genetically-detoxified **mutant** of pertussis toxin is new. Vaccine against *Bordetella pertussis* comprises an effective amt. of the **mutant**, or its toxoid, and an acceptable carrier. Conjugate vaccine comprises the **mutant** as carrier protein for a hapten, polysaccharide or polypeptide. New strains of *Bordetells pertussis* are characterised by either (i) the absence of the toxin operon and foreign DNA and by the ability to be grown in the absence of antibiotics to produce *B. pertussis* antigens free of **pertussis toxin**; or (ii) the toxin operon having been replaced by a **mutant** gene formed by site-directed **mutagenesis** of at least one specific amino acid residue responsible for pertussis toxin toxicity. Native *Bordetella pertussis* 10536 TOX operon is new having a given nucleotide sequence and structural gene translation.

ADVANTAGE - Residual toxicity is 1% or less, pref. less than 0.5% of that of the native toxin. Genetic detoxification avoids the problems of chemical detoxification using e.g. formaldehyde, glutaraldehyde or H<sub>2</sub>O<sub>2</sub>, i.e. obtaining a balance between sufficient detoxification and loss of potency.

0/20

Dwg.0/20

ABEQ US 5085862 A UPAB: 19930923

Immunoprotective genetically detoxified **mutant** of pertussis holotoxin is formed by genetic modification of the A portion (S1 subunit) and/or B portion of the holotoxin.

Pref. a single amino acid in the native holotoxin is removed or replaced e.g. glu-129 is removed and opt. replaced by gly, or arg-58 is replaced by glu, etc. **Mutant** has residual toxicity, less than 0.5% of native toxin.

ADVANTAGE - Has decreased histamine sensitivity in a vaccine against *Bordetella pertussis*.

ABEQ US 5221618 A UPAB: 19931116

Strain of *Bordetella* capable of expressing an immunoprotective genetically-detoxified **mutant** of pertussis holotoxin.

Toxin operon has been replaced by a **mutant** operon formed by **mutagenesis** of a nucleotide sequence encoding at least one specific aminoacid residue which contributes to pertussis toxin toxicity.

Also claimed is a **method** of producing an immunoprotective, genetically-detoxified pertussis holotoxin **mutant**.

USE/ADVANTAGE - As a vaccine against pertussis.

Dwg.0/10

ABEQ US 5244657 A UPAB: 19931123

Immunoprotective genetically-detoxified **mutant** of pertussis halo-toxin has a single amino acid in its S1 sub-unit of the native form replaced, i.e. arg9 by lys9.

**Mutant** has residual toxicity less than 0.5% of native toxic. Prodn. comprises site-directed **mutagenesis** of native pertussis toxin gene. **Mutant** has decreased histamine sensitivity activity.

USE - In prepn. of safe, immunogenic and efficacious vaccine for protection against pertussis.

Dwg.0/15

ABEQ US 5332583 A UPAB: 19940907

Vaccine against *Bordetella* pertussis comprises a **mutant** of pertussis holotoxin (where at least one amino acid is removed or replaced) and at least one other pertussis antigen e.g. agglutinogens, FHA or 69 kD membrane protein.

ADVANTAGE - Vaccine is safe and effective.

Dwg.0/29

ABEQ US 5358868 A UPAB: 19941212

Strain of *Bordetella* has the toxin operon replaced by a **mutant** gene formed by site-directed **mutagenesis** of a sequence encoding the S1 and S3 subunit of pertussis holotoxin. Has ATCC Nos. 53833, 53834, 53836, 53837, 53974, 53975 or 53976.

USE/ADVANTAGE - Prepn. of a vaccine against pertussis. Vaccine is safe.

Dwg.0/29

ABEQ US 5433945 A UPAB: 19950904

Immunoprotective genetically-detoxified **mutant** of pertussis holotoxin has multiple amino acids in the native toxin replaced or removed. Specific examples include Arg-58 and Gly-129 replaced by Glu-58 and Gly-129, and Arg-9 and Glu-129 replaced by Ly's-9 and Gly-129 in the SI subunit. **Mutants** have a residual toxicity of less than 0.5%.

USE/ADVANTAGE - Used as a vaccine against pertussis. Retains immunological properties without having undesirable side effects. decreased histamine sensitivity.

Dwg.0/15

ABEQ EP 322115 B UPAB: 19960405

A **mutant** pertussis holotoxin obtained by expression of a tox operon encoding the holotoxin which has been **mutated** by site-directed **mutagenesis** of at least one codon encoding at least one functional amino acid within native pertussis holotoxin including at least one of (A1) ARG9, ARG13 and GLU129, to effect removal or replacement of said at least one functional amino acid and to genetically detoxify said holotoxin to a residual toxicity of 1% or less while retaining immunoprotective properties.

Dwg.0/10

09/825769

ACCESSION NUMBER: 1989-085926 JAPIO  
TITLE: MUTANT OF BORDETELLA PERTUSSIS  
INVENTOR: SATO YUJI; SATO HIROKO; YOSHIDA IWAO; IMAIZUMI ATSUSHI  
PATENT ASSIGNEE(S): TEIJIN LTD  
KOKURITSU YOBOU EISEI KENKYUSHO  
HANDAI BISEIBUTSUBIYOU KENKYUKAI  
PATENT INFORMATION:

PATENT NO	KIND	DATE	ERA	MAIN IPC
JP 01085926	A	19890330	Heisei	A61K039-10

APPLICATION INFORMATION

STN FORMAT: JP 1988-144630 19880614  
ORIGINAL: JP63144630 Showa  
PRIORITY APPLN. INFO.: JP 1987-155577 19870624  
SOURCE: PATENT ABSTRACTS OF JAPAN (CD-ROM), Unexamined Applications, Vol. 1989

AN 1989-085926 JAPIO

AB PURPOSE: To provide a **mutant** of *Bordetella pertussis* phase-I, free from pertussis toxicity and **producing** a **pertussis toxin** protein devoid of a part of subunit capable of deriving an antibody neutralizing the biological activity of pertussis toxin.

CONSTITUTION: Phase-I cell of *Bordetella pertussis* is cultured by conventional **method** and washed under centrifugal treatment. The obtained bacterial cells are suspended in a tris-maleic acid buffer solution at a concentration of 10<sup>10</sup> mol./ml, added with 25~50μg/ml of nitrosoureasine as a **mutagenic** agent and shaken for 60min. The product is subjected to centrifugal washing in a liquid medium, properly diluted and cultured on a solid medium by plate culture. A colony on the plate is picked up, inoculated in a liquid medium, cultured by shaking culture and screened with respect to the supernatant liquid of the cultured product to obtain a 79G strain (FERM BP-1902) which is inert to the clustering of CHO cell and reactive with a polyclonal antibody of pertussis toxin. The 79G strain is cultured in a medium added with a cyclodextrin and a pertussis toxin or protein devoid of a part of subunit, especially subunit S1 is separated from the cultured product.

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L13 ANSWER 10 OF 11 MEDLINE DUPLICATE 5

ACCESSION NUMBER: 90049157 MEDLINE  
DOCUMENT NUMBER: 90049157 PubMed ID: 2683073  
TITLE: **Mutants** of pertussis toxin suitable for vaccine development.  
AUTHOR: Pizza M; Covacci A; Bartoloni A; Perugini M; Nencioni L; De Magistris M T; Villa L; Nucci D; Manetti R; Bugnoli M; +  
CORPORATE SOURCE: Sclavo Research Center, Siena, Italy.  
SOURCE: SCIENCE, (1989 Oct 27) 246 (4929) 497-500.  
Journal code: 0404511. ISSN: 0036-8075.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals

09/825769

ENTRY MONTH: 198912  
ENTRY DATE: Entered STN: 19900328  
Last Updated on STN: 19900328  
Entered Medline: 19891204

AB Immunization with chemically detoxified pertussis toxin can prevent severe whooping cough with an efficacy similar to that of the cellular pertussis vaccine, which normally gives unwanted side effects. To avoid the reversion to toxicity and the loss of immunogenicity that may follow chemical treatment of pertussis toxin, inactive toxins were constructed by genetic manipulation. A number of genetically engineered alleles of the pertussis toxin genes, constructed by replacing either one or two key amino acids within the enzymatically active S1 subunit, were introduced into the chromosome of strains of *Bordetella pertussis*, *B. parapertussis*, and *B. bronchiseptica*. These strains **produce mutant pertussis toxin** molecules that are nontoxic and immunogenic and that protect mice from the intracerebral challenge with virulent *Bordetella pertussis*. Such molecules are ideal for the development of new and safer vaccines against whooping cough.

L13 ANSWER 11 OF 11 WPIDS (C) 2002 THOMSON DERWENT  
ACCESSION NUMBER: 1989-001225 [01] WPIDS  
DOC. NO. CPI: C1989-000473  
TITLE: *Bordetella pertussis* variant producing **mutant** toxin protein - lacking at least sub unit 1, used in vaccine prepn., e.g. against whooping cough.  
DERWENT CLASS: B04 D16  
INVENTOR(S): IMAIZUMI, A; SATO, H; SATO, Y; YOSHIDA, I  
PATENT ASSIGNEE(S): (OSAB-N) OSAKA BISEIBUTSUBYO ZH; (REMI-N) RES FOUND MICROBIAL DISE; (TEIJ) TEIJIN LTD; (NAHE-N) NAT INST OF HEALTH JAPAN; (OSAU) UNIV OSAKA  
COUNTRY COUNT: 15  
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
EP 296765	A	19881228 (198901)*	EN	12	
	R: AT BE CH DE ES FR GB GR IT LI NL SE				
AU 8818295	A	19890302 (198918)			
JP 01085926	A	19890330 (198919)			
US 5223255	A	19930629 (199327)		9	
EP 296765	B1	19940608 (199422)	EN	13	
	R: AT BE CH DE ES FR GB GR IT LI NL SE				
DE 3889975	G	19940714 (199428)			

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
EP 296765	A	EP 1988-305530	19880617
JP 01085926	A	JF 1988-144630	19880614
US 5223255	Cont of	US 1988-209599	19880622
		US 1991-774637	19911011
EP 296765	B1	EP 1988-305530	19880617
DE 3889975	G	DE 1988-3889975	19880617
		EP 1988-305530	19880617

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FILING DETAILS:

PATENT NO	KIND	PATENT NO
DE 3889975	G Based on	EP 296765
PRIORITY APPLN. INFO: JP 1987-155577 19880614		19870624; JP 1988-144630
AN	1989-001225 [01] WPIDS	
AB	EP 296765 A UPAB: 19930923	A <i>Bordetella pertussis</i> variant which <b>produces</b> a <i>pertussis toxin mutant</i> protein partially devoid of at least subunit 1 and the protein are claimed. The variant is deposited as FERM BP-1902. Also claimed is a <b>method</b> for producing the protein by culturing the variant in a medicine contg. cyclodextrin or derivs., and a vaccine prep. using the <b>mutant</b> protein. USE/ADVANTAGE - Pertussis toxin is a major pathogenic factor in whooping cough and so is an important protective antigen in pertussis vaccine. Allows the prodn. of a B pertussis variant, which produces a protein partially devoid of subunits, esp. at least 51, which can be harvested from the culture of the variant.
	0/3	
ABEQ	US 5223255 A UPAB: 19931116	<i>Bordetella pertussis</i> variant <b>produces</b> a <i>pertussis toxin</i> protein lacking ADP-ribosyltransferase activity associated with the S1 subunit. Variant is deposited in the Fermentation Research Institute with International Deposition No. FERM BP-1902. Also claimed are the <b>mutant</b> protein and a vaccine contg. the protein. USE/ADVANTAGE - As a pertussis vaccine without toxic activity.
	Dwg.0/3	
ABEQ	EP 296765 B UPAB: 19940722	A <i>Bordetella pertussis</i> variant which <b>produces</b> a <i>pertussis toxin mutant</i> protein partially devoid of subunits, which toxin is devoid of at least subunit S1. Dwg.0/3
<del>FILE "HCAPLUS"</del> ENTERED AT 11:25:11 ON 31 OCT 2002		
L1	12 SEA FILE=REGISTRY ABB=ON PLU=ON ("PERTUSSIS TOXIN"/CN OR "PERTUSSIS TOXIN (BORDETELLA PERTUSSIS 10536 CLONE J-169-1 S1 SUBUNIT)"/CN OR "PERTUSSIS TOXIN (BORDETELLA PERTUSSIS 10536 CLONE J-169-1 S2 SUBUNIT)"/CN OR "PERTUSSIS TOXIN (BORDETELLA PERTUSSIS 10536 CLONE J-169-1 S3 SUBUNIT)"/CN OR "PERTUSSIS TOXIN (BORDETELLA PERTUSSIS TOXIN SUBUNIT S1 VARIANT 1)"/CN OR "PERTUSSIS TOXIN (BORDETELLA PERTUSSIS TOXIN SUBUNIT S1 VARIANT 2)"/CN OR "PERTUSSIS TOXIN (SYNTHETIC BORDETELLA BRONCHISEPTICA-BORDETELLA PERTUSSIS SUBUNIT S1)"/CN OR "PERTUSSIS TOXIN (SYNTHETIC BORDETELLA BRONCHISEPTICA-BORDETELLA PERTUSSIS SUBUNIT S2)"/CN OR "PERTUSSIS TOXIN (SYNTHETIC BORDETELLA BRONCHISEPTICA-BORDETELLA PERTUSSIS SUBUNIT S3)"/CN OR "PERTUSSIS TOXIN (SYNTHETIC BORDETELLA BRONCHISEPTICA-BORDETELLA PERTUSSIS SUBUNIT S4)"/CN OR "PERTUSSIS TOXIN (SYNTHETIC BORDETELLA BRONCHISEPTICA-BORDETELLA PERTUSSIS SUBUNIT S5)"/CN OR "PERTUSSIS TOXIN (SYNTHETIC)"/CN)	
L2	1 SEA FILE=REGISTRY ABB=ON PLU=ON "BORDETELLA BRONCHISEPT	

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ICA"/CN  
L3 13 SEA FILE=REGISTRY ABB=ON PLU=ON L1 OR L2  
L4 9821 SEA FILE=HCAPLUS ABB=ON PLU=ON L3 OR PT(10A)TOXIN OR  
PERTUSSIS TOXIN  
L14 1472 SEA FILE=HCAPLUS ABB=ON PLU=ON (KNOCKOUT OR KNOCK  
OUT)(S)(MUTAT? OR MUTANT OR MUTAGEN? OR POLYMORPH? OR  
POLY MORPH?)  
L15 2 SEA FILE=HCAPLUS ABB=ON PLU=ON L4 AND L14

L16 2 L15 NOT L9

L16 ANSWER 1 OF 2 HCAPLUS COPYRIGHT 2002 ACS  
ACCESSION NUMBER: 1999:700149 HCAPLUS  
DOCUMENT NUMBER: 132:147072  
TITLE: Effect of knockout AT1a receptor gene on trans  
plasma membrane calcium influx in aortic smooth  
muscle cells  
AUTHOR(S): Zhu, Zhiming; Zhu, Shanjun; Hu, Houxiang  
CORPORATE SOURCE: Hypertension Center, Daping Hospital, The Third  
Military Medical University, Chungking, 400042,  
Peop. Rep. China  
SOURCE: Zhonghua Yixue Zazhi (1999), 79(9), 661-663  
CODEN: CHHTAT; ISSN: 0376-2491  
PUBLISHER: Zhonghua Yixue Zazhi  
DOCUMENT TYPE: Journal  
LANGUAGE: Chinese  
AB The mechanism of angiotensin II receptor subtype (AT1a)  
mutant on angiotensin II (Ang II)-mediated transmembrane  
Ca2+ influx in aortic smooth muscle cells (SMCs) from AT1a  
knockout and wild type mice was studied. Aortic SMCs were  
isolated and cultured. Intracellular free Ca concn. [Ca2+]i was  
measured using Fura-2/AM fluorescence technique. Ang II caused a  
marked increase in [Ca2+]i in both cell types (AT1a group: 204  
nmol/L vs. 108 nmol/L; control: 194 nmol/L vs. 110 nmol/L).  
Administration of both the calcium channel blocker nifedipine and  
GTP-.gamma.s significantly inhibited the Ang II effect; in contrast,  
application of pertussis toxin (PTX) activated  
the Ang II-mediated Ca2+ influx. The response of AT1a knockout  
cells was more sensitive to nifedipine and was enhanced by PTX.  
Ca2+ influx induced by Ang II can be regulated by PTX insensitive  
and non-Gi protein in AT1a knockout cells.

L16 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2002 ACS  
ACCESSION NUMBER: 1998:380151 HCAPLUS  
DOCUMENT NUMBER: 129:131899  
TITLE: Natural competence for DNA transformation in  
*Helicobacter pylori*: identification and genetic  
characterization of the comB locus  
AUTHOR(S): Hofreuter, Dirk; Odenbreit, Stefan; Henke,  
Gabriele; Haas, Rainer  
CORPORATE SOURCE: Infektionsbiologie, Max-Planck-Institut for  
Biologie, Tobingen, D-72076, Germany  
SOURCE: Molecular Microbiology (1998), 28(5), 1027-1038  
CODEN: MOMIEE; ISSN: 0950-382X  
PUBLISHER: Blackwell Science Ltd.  
DOCUMENT TYPE: Journal

09/825769

LANGUAGE: English

AB The Gram-neg. bacterial pathogen Helicobacter pylori, an important etiol. agent of gastroduodenal disease in humans, belongs to a group of bacterial species displaying competence for genetic transformation. Here, the authors describe the comb gene locus of H. pylori involved in DNA transformation competence. It consists of a cluster of four tandemly arranged genes with partially overlapping open reading frames, orf2, comB1, comB2 and comB3, constituting a single transcriptional unit. Orf2 encodes a 37-amino-acid peptide carrying a signal sequence, whereas comB1, comB2 and comB3 produce 29kDa, 38kDa and 42kDa proteins, resp., as demonstrated by immunoblotting with specific antisera. For Orf2 and ComB1, no homologous proteins were identified in the database. For ComB3, the best homologies were found with TraS/TraB from the Pseudomonas aeruginosa conjugative plasmid RP1 and Trb1 of plasmid RP4, VirB10 from the Ti plasmid of Agrobacterium tumefaciens and PtIG, a protein involved in secretion of pertussis toxin of Bordetella pertussis. Defined transposon knock-out mutants in individual comb genes resulted in transformation-defective phenotypes ranging from a 90% redn. to a complete loss of the natural transformation efficiency. The comB2 and comB3 genes show homol. to HP0528 and HP0527, resp., located on the cagII pathogenicity island of H. pylori strain 26695.

~(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO, TOXCENTER' ENTERED AT 11:26:55 ON 31 OCT 2002)

L17 13 S L15

L18 12 S L17 NOT L12

L19 4 DUP REM L18-(8 DUPLICATES REMOVED)

L19 ANSWER 1 OF 4 MEDLINE DUPLICATE 1

ACCESSION NUMBER: 2000094958 MEDLINE

DOCUMENT NUMBER: 20094958 PubMed ID: 10629036

TITLE: Normal hematopoiesis and inflammatory responses despite discrete signaling defects in Galpha15 knockout mice.

AUTHOR: Davignon I; Catalina M D; Smith D; Montgomery J; Swantek J; Croy J; Siegelman M; Wilkie T M

CORPORATE SOURCE: Pharmacology Department, UT Southwestern, Dallas TX 75235-9041, USA.

CONTRACT NUMBER: 5-T32-GM07062 (NIGMS)  
DK47890 (NIDDK)

SOURCE: MOLECULAR AND CELLULAR BIOLOGY, (2000 Feb) 20 (3) 797-804.

Journal code: 8109087. ISSN: 0270-7306.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200002

ENTRY DATE: Entered STN: 20000229

Last Updated on STN: 20000229

Entered Medline: 20000214

AB Galpha15 activates phospholipase C $\beta$  in response to the greatest variety of agonist-stimulated heptahelical receptors among the four Gq class G-protein alpha subunits expressed in mammals. Galpha15 is primarily expressed in hematopoietic cells in fetal and adult mice. We disrupted the Galpha15 gene by homologous recombination in

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embryonic stem cells to identify its biological functions. Surprisingly, hematopoiesis was normal in Galphai5(-/-) mice, Galphai5(-/-) Galphaq(-/-) double-knockout mice (which express only Galphai1 in most hematopoietic cells), and Galphai1(-/-) mice, suggesting functional redundancy in Gq class signaling. Inflammatory challenges, including thioglycolate-induced peritonitis and infection with *Trichinella spiralis*, stimulated similar responses in Galphai5(-/-) adults and wild-type siblings. Agonist-stimulated Ca(2+) release from intracellular stores was assayed to identify signaling defects in primary cultures of thioglycolate-elicited macrophages isolated from Galphai5(-/-) mice. C5a-stimulated phosphoinositide accumulation and Ca(2+) release was significantly reduced in Galphai5(-/-) macrophages. Ca(2+) signaling was abolished only in mutant cells pretreated with pertussis toxin, suggesting that the C5a receptor couples to both Galphai5 and Galphai1 in vivo. Signaling evoked by other receptors coupled by Gq class alpha subunits appeared normal in Galphai5(-/-) macrophages. Despite discrete signaling defects, compensation by coexpressed Gq and/or Gi class alpha subunits may suppress abnormalities in Galphai5-deficient mice.

L19 ANSWER 2 OF 4 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 2001:92530 BIOSIS

DOCUMENT NUMBER: PREV200100092530

TITLE: Functional knockout of a dopamine-activated K<sup>+</sup>-channel reverses dopaminergic inhibition of acute prolactin release.

AUTHOR(S): Horel, J. S. (1); Welling, P. A.; O'Neill, T. J.; Gregerson, K. A.

CORPORATE SOURCE: (1) Univ of Maryland Baltimore Sch of Med, Baltimore, MD USA

SOURCE: Society for Neuroscience Abstracts, (2000) Vol. 26, No. 1-2, pp. Abstract No.-627.3. print.  
Meeting Info.: 30th Annual Meeting of the Society of Neuroscience New Orleans, LA, USA November 04-09, 2000 Society for Neuroscience  
. ISSN: 0190-5295.

DOCUMENT TYPE: Conference

LANGUAGE: English

SUMMARY LANGUAGE: English

AB The major regulator of prolactin (PRL) release in vivo is that of hypothalamic dopamine (DA), which exerts a tonic inhibitory tone on PRL secretion. While it is known that this inhibition is mediated via the D2 receptors on pituitary lactotropes, the signaling events responsible for the inhibition of acute PRL release remains an area of debate. We have characterized a pertussis-toxin sensitive, inward rectifier K<sup>+</sup> channel in lactotropes that appears to be a critical mediator of DA's inhibitory effect on acute release. Through activation of this G-protein gated channel, DA hyperpolarizes the lactotrope membrane, closing voltage-gated Ca<sup>2+</sup> channels and reducing the driving force for PRL release. We have recently identified a GIRK1/GIRK4 heteromultimeric channel (G protein gated, Inward Rectifier K<sup>+</sup> channel) in the anterior pituitary as the probable DA-activated K<sup>+</sup> channel (KDA). Based on this molecular identification, we have begun to directly investigate the physiological role of the channel in the regulation of PRL release through the functional "knockout" of the channel in vitro. To this end, we constructed a dominant negative GIRK1

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**mutant** (G1AAA) that blocks wild type channel activity. Using an adenoviral expression system, this **mutant** was introduced into dissociated pituitary cells to block KDA at the protein level. Varying infection dose revealed that expression of G1AAA could reverse dopaminergic inhibition of acute PRL release in a dose dependent manner. These data highlight the KDA channel as a physiologically important effector in the dopaminergic inhibition of acute PRL release.

L19 ANSWER 3 OF 4 TOXCENTER COPYRIGHT 2002 ACS  
ACCESSION NUMBER: 1999:202982 TOXCENTER  
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DOCUMENT NUMBER: CA13212147072C  
TITLE: Effect of knockout AT1a receptor gene on trans plasma membrane calcium influx in aortic smooth muscle cells  
AUTHOR(S): Zhu, Zhiming; Zhu, Shanjun; Hu, Houxiang  
CORPORATE SOURCE: Hypertension Center, Daping Hospital, The Third Military Medical University, Chungking, 400042, Peop. Rep. China.  
SOURCE: Zhonghua Yixue Zazhi, (1999) Vol. 79, No. 9, pp. 661-663.  
CODEN: CHHTAT. ISSN: 0376-2491.

COUNTRY: CHINA  
DOCUMENT TYPE: Journal  
FILE SEGMENT: CAPLUS  
OTHER SOURCE: CAPLUS 1999:700149  
LANGUAGE: Chinese  
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AB The mechanism of angiotensin II receptor subtype (AT1a) **mutant** on angiotensin II (Ang II)-mediated transmembrane Ca<sup>2+</sup> influx in aortic smooth muscle cells (SMCs) from AT1a **knockout** and wild type mice was studied. Aortic SMCs were isolated and cultured. Intracellular free Ca concn. [Ca<sup>2+</sup>]<sub>i</sub> was measured using Fura-2/AM fluorescence technique. Ang II caused a marked increase in [Ca<sup>2+</sup>]<sub>i</sub> in both cell types (AT1a group: 204 nmol/L vs. 108 nmol/L; control: 194 nmol/L vs. 110 nmol/L). Administration of both the calcium channel blocker nifedipine and GTP-.gamma.s significantly inhibited the Ang II effect; in contrast, application of **pertussis toxin** (PTX) activated the Ang II-mediated Ca<sup>2+</sup> influx. The response of AT1a knockout cells was more sensitive to nifedipine and was enhanced by PTX. Ca<sup>2+</sup> influx induced by Ang II can be regulated by PTX insensitive and non-Gi protein in AT1a knockout cells.

L19 ANSWER 4 OF 4 MEDLINE DUPLICATE 2  
ACCESSION NUMBER: 1998326821 MEDLINE  
DOCUMENT NUMBER: 98326821 PubMed ID: 9663688  
TITLE: Natural competence for DNA transformation in Helicobacter pylori: identification and genetic characterization of the comB locus.  
AUTHOR: Hofreuter D; Odenbreit S; Henke G; Haas R  
CORPORATE SOURCE: Max-Planck-Institut fur Biologie, Abteilung, Infektionsbiologie, Tubingen, Germany.  
SOURCE: MOLECULAR MICROBIOLOGY, (1998 Jun) 28 (5) 1027-38.  
PUB. COUNTRY: Journal code: 8712028. ISSN: 0950-382X.  
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DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-AJ132366  
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AB The gram-negative bacterial pathogen *Helicobacter pylori*, an important aetiological agent of gastroduodenal disease in humans, belongs to a group of bacterial species displaying competence for genetic transformation. Here, we describe the comB gene locus of *H. pylori* involved in DNA transformation competence. It consists of a cluster of four tandemly arranged genes with partially overlapping open reading frames, orf2, comB1, comB2 and comB3, constituting a single transcriptional unit. Orf2 encodes a 37-amino-acid peptide carrying a signal sequence, whereas comB1, comB2 and comB3 produce 29 kDa, 38 kDa and 42 kDa proteins, respectively, as demonstrated by immunoblotting with specific antisera. For Orf2 and ComB1, no homologous proteins were identified in the database. For ComB3, the best homologies were found with TraS/TraB from the *Pseudomonas aeruginosa* conjugative plasmid RP1 and TrbI of plasmid RP4, VirB10 from the Ti plasmid of *Agrobacterium tumefaciens* and PtlG, a protein involved in secretion of *pertussis toxin* of *Bordetella pertussis*. Defined transposon **knock-out mutants** in individual comB genes resulted in transformation-defective phenotypes ranging from a 90% reduction to a complete loss of the natural transformation efficiency. The comB2 and comB3 genes show homology to HP0528 and HP0527, respectively, located on the cagII pathogenicity island of *H. pylori* strain 26695.

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